Journal of Heredity 2007:98(3):211–220
doi:10.1093/jhered/esm019
Advance Access publication May 16, 2007

Mutation in *CEP290* Discovered for Cat Model of Human Retinal Degeneration

MARILYN MENOTTI-RAYMOND, VICTOR A. DAVID, ALEJANDRO A. SCHÄFFER, ROBERT STEPHENS, DAVID WELLS, RAJENDRA KUMAR-SINGH, STEPHEN J. O'BRIEN, AND KRISTINA NARFSTRÖM

From the Laboratory of Genomic Diversity, National Cancer Institute-Frederick, Frederick, MD 21702 (Menotti-Raymond, David, Wells, O'Brien); National Center for Biotechnology Information, National Institutes of Health, Department of Health and Human Services, Bethesda, MD 20894 (Schäffer); Advanced Biomedical Computing Center, National Cancer Institute-Frederick, Frederick, MD 21702 (Stephens); Department of Ophthalmology, Tufts University School of Medicine, Boston, MA 02111 (Kumar-Singh); and Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia, Columbia, MO 65211 (Narfström); and Department of Ophthalmology, Mason Eye Institute, University of Missouri-Columbia, Columbia, MO 65211 (Narfström).

Address correspondence to M. Menotti-Raymond at the address above, or e-mail: raymond@ncifcrf.gov.

Abstract

A mutation in the *CEP290* gene is reported in a cat pedigree segregating for autosomal recessive (AR) late-onset photoreceptor degeneration (rdAc). An initial screen of 39 candidate genes and genomic locations failed to detect linkage to cat rdAc. Linkage was ultimately established on cat B4 with 15 simple tandem repeat markers (logarithm of odds [LOD] range 4.83–15.53, $\Theta = 0.0$), in a region demonstrating conserved synteny to human chromosome 12, 84.9–90.63 Mb. The sequence of 10 genes with feline retinal expression was examined in affected and unaffected individuals. A single-nucleotide polymorphism was characterized in intron 50 of *CEP290* (IVS50 + 9T>G) that creates a strong canonical splice donor site, resulting in a 4-bp insertion and frameshift in the mRNA transcript, with subsequent introduction of a stop codon and premature truncation of the protein. A population genetic survey of 136 cats demonstrated that the rdAc mutation is in low frequency in Abyssinian populations (0.13, Sweden; 0.07, United States) and absent in breeds of non-Abyssinian heritage. Mutations in *CEP290* have recently been shown to cause two human diseases, Joubert syndrome, a syndromic retinal degeneration, and Leber's congenital amaurosis, an AR early-onset retinal dystrophy. Human AR retinitis pigmentosa is among the most common causes of retinal degeneration and blindness, with no therapeutic intervention available. This identification of a large animal model for human retinal blindness offers considerable promise in developing gene-based therapies.

In humans, autosomal recessive retinitis pigmentosa (ARRP) is a genetically and clinically heterogeneous and progressive degenerative disorder of the retina, primarily affecting the rod photoreceptors. Clinically, RP is manifested as night blindness, loss of peripheral vision and eventually the loss of central vision, with severe visual handicap in adulthood. There are no available therapeutic interventions. Twenty mapped and identified genes and 5 additional genomic regions (no gene established) are identified as causative of RP (http://www.sph.uth.tmc.edu/retnet/). No single gene accounts for more than 10% of RP incidence, and for approximately 60% of RP cases, the causative gene has not been identified.

An important cat model of human RP has been maintained for more than 25 years by K.N., which segregates for AR retinal degeneration. This progressive retinal atrophy affecting Abyssinian cats is designated (rdAc). Affected cats have normal vision at birth but develop early funduscopic changes at the age of 1.5–2 years (Narfström 1985a). Early functional ophthalmic changes are demonstrated by electroretinographic (ERG) studies: at the age of 7 months, affected cats may be differentiated from unaffected by significantly reduced ERG a-wave amplitudes, shown to parallel a reduction in retinal oxygen tension (Kang Derwent et al. 2006). Morphological changes are initially observed in individual rod photoreceptor outer segments from age 5–8 months (Narfström and Nilsson 1989). A disorganization and disruption of rod outer segment lamellar discs are seen usually with vacuoles at the base of the outer segment near the connecting cilium. With progression of disease, entire rod outer segments become disrupted and solitary rods or rods in patches degenerate. Cones are similarly involved later in the disease process, and complete photoreceptor degeneration and blindness is observed at the end stage, usually at the age of 3–5 years (Narfström 1985b). Many similarities have been found between the Abyssinian cat retinal degeneration and human RP (Jacobson et al. 1989; Narfström et al. 1989). Prior

molecular genetic analyses examining retinally expressed sequence of cDNA generated for candidate genes phosducin and peripherin detected no abnormalities in rdAc individuals (Snyder et al. 1990; Gorin et al. 1993, 1995)]. Additionally, report was made of transcript abundance of several retinally expressed genes compared with age-matched controls (Gorin et al. 1992). No systemic abnormalities have been noted in the disease (Narfström 1985b).

We report the disease mutation for rdAc, a single-nucleotide polymorphism (SNP) in an intronic region of the *CEP290* gene, which creates a strong canonical splice donor site, resulting in a 4-bp insertion and frameshift in the mRNA transcript, with subsequent premature truncation of the protein. Identification of the gene defect in a large animal model for human retinal blindness offers considerable promise for the development of therapeutic intervention.

Materials and Methods

Animals

A 5.5-year-old, blind, purebred male Abyssinian cat, used for breeding and show purposes, was initially discovered in Sweden, 1977. His offspring and other privately owned Abyssinians from Sweden were thereafter screened in the country for clinical signs of retinal disease using ophthalmoscopy. A high frequency of a progressive retinal degeneration was found in the breed (Narfström 1983) and further characterized through clinical and laboratory studies (Narfström et al. 1985; Narfström 1985a; Narfström and Nilsson 1986, 1989). A breeding colony of affected cats was established in Sweden and moved to the University of Missouri-Columbia in 2001. Outcrossed, natural matings with European short-haired cats were performed in order to increase heterozygosity in the colony, and 55 backcrossed cats were generated. Cats were diagnosed as affected or unaffected by bilateral, full-field ERG recordings at age 7–12 months, using a standardized protocol developed for the study (Vaegan and Narfström 2004; Hyman et al. 2005). Clinical status of each cat was verified within another 6–12 months through funduscopic examinations and follow-up ERGs according to a University of Missouri approved Animal Use and Care Protocol (no. 3689). Blood and tissue samples were obtained for the molecular genetic studies.

Genotyping and Marker Development

Amplification of 88 short tandem repeats (STRs) was performed by touchdown polymerase chain reaction (PCR) reactions as described by Menotti-Raymond et al. (Menotti-Raymond et al. 2005); PCR products were fluorescently labeled as described by Boutin-Ganache et al. (2001). New STRs were designed for candidate loci/genomic regions both in the initial candidate screen and later for fine mapping of rdAc in the implicated genomic region by identifying STRs in orthologous cat-coding sequence in the growing database of unassembled and unannotated *Felis catus* 2X whole-genome shotgun sequences (http://www.ncbi.nlm.nih.gov/BLAST) by discontiguous MEGABLAST search (Zhang et al. 2000) using human or dog genes plus flanking sequences (http://www.ncbi.nlm.nih.gov/BLAST). Cat traces identified were then screened for STRs by REPEAT FINDER (http://www.genet.sickkids.on.ca/~ali/repeatfinder.html), and PCR primers were designed with PRIMER 3.0 (Rozen and Skaletsky 2000) (Table A2 and Table A3).

Linkage Analysis

Linkage analysis computations were performed with SUPERLINK (Fishelson and Geiger 2002, 2004). For the results mentioned here, we modeled rdAc as a fully penetrant, AR disease with disease allele frequency 0.001. At early stages, we considered the possibility of less than full penetrance due to the late age of onset, but this only dampened the positive LOD scores. Marker allele frequencies were set all equal. Marker order was checked both with respect to the cat radiation hybrid map and using multipoint, marker-only analysis on the rdAc pedigree data.

RNA Extraction and Generation of cDNAs

Retinal neuronal and pigment epithelial tissues harvested from normal and rdAc-affected cats were stored at −80 °C in RNAlater (Ambion, Austin, TX). Reverse transcriptase (RT)–PCR was performed with the SuperScript III One-Step RT-PCR kit (Invitrogen, Carlsbad, CA). RNA was extracted using the RNAqueous-4 PCR kit (Ambion) RT-PCR products were visualized on 2% agarose gels and sequenced as previously described (Ishida et al. 2006). When multiple bands were present, individual bands were isolated by touching each band separately with a toothpick and placing the toothpick in a microcentrifuge tube with 10 μl of water. Then 1 μl of this solution was PCR amplified using a touchdown procedure using PCR reaction conditions and cycling times as previously described (Menotti-Raymond et al. 2005). The PCR primers used for amplification of the *CEP290* cDNA are listed in Table A4. The initial RT-PCR of *CEP290* did not result in any products detectable on an agarose gel, so 1 μl of the products was subjected to touchdown PCR amplification. All the other cDNA's we amplified (Table 4) did not require this additional amplification. DNA sequences were aligned in SEQUENCHER VERSION 4.5 (Gene Codes Corp. Ann Arbor, MI).

Gene-Centric Cat Genome Assemblies

Because the assembled cat genomic sequence was not available at the onset of this project, we produced cDNA assemblies of the genes from the identified genomic region. These assembled regions were then scanned for mutations when compared with their human and canine paralogous genes. For each gene in the region, the mRNA of the gene was used to identify all cat traces with homology to that mRNA using BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). The resulting bank of traces was then trimmed to include only the mRNA homologous stretches (using FASTACMD) (http://www.ncbi.nlm.nih.gov/BLAST/) (Altschul et al. 1990) and the resulting sequences were assembled into contigs using PHRAP (http://www.phrap.org/). An alignment

of the resulting contigs to all available mRNAs for the gene from different organisms was built using information from homologene (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=homologene). Finally, the alignment was translated and examined for amino acid changes and compared with the palette of all known variant residues from a similar multiple sequence alignment of the protein sequences. Differences in amino acids and also frameshifts and other alterations were identified from this analysis. In a parallel approach, mRNA sequences from Refseq (http://www.ncbi.nlm.nih.gov/RefSeq/) (Pruitt et al. 2005) were aligned to the assembled cat genomic sequences produced from the Broad Institute/National Human Genome Research Institute/Laboratory of Genomic Diversity/Advanced Biomedical Computing Center consortium using the GMAP program (Wu and Watanabe 2005). For some of the mRNAs in the identified region, because of missing exons in the 2X assembly, matches were not identified. To further elucidate the alignments for this subset of mRNAs, the mRNAs were broken down into individual exons (with some flanking intronic bases included) from the genomic contigs that contained them from either dog or human. The resulting exons were then also scanned using the GMAP program. The computed alignments from GMAP were then screened for amino acid mutations or other alterations. Identified alterations were then assessed in the affected/unaffected cat context using genomic PCR (or RT-PCR).

In *CEP290* intron 50, we observed a single-base substitution (IVS50 + 9T>G) that generates a strong canonical splice donor site 4 bp downstream of the noncanonical splice donor site observed in wild-type individuals. Canonical splice sites have been described/defined by Burset et al. (2000) from the analysis of a set of 43 337 splice junction pairs extracted from mammalian GenBank annotated genes. Of these pairs, 98.71% contained the dinucleotides GT and AG for donor and acceptor sites, respectively, which were deemed "canonical." In rdAc-affected individuals, the single-base substitution (IVS50 + 9T>G) generates a canonical splice site "GT," 4 bp downstream of the noncanonical splice donor "GC" site.

Amino Acid Alignment of Feline CEP290

Cat CEP290 amino acid sequence, as translated from cDNA generated from RNA extracted from retinal neuronal tissue from two normal individuals, was aligned to human, rat, and dog CEP290 sequences reported in GenBank using CLUSTALW (Chenna et al. 2003).

Population Genetic Survey of *CEP290* Mutation in Abyssinian/Somali Breeds and 20 Additional Cat Breeds

A sample set of 56 Abyssinian and 23 Somali (long-haired Abyssinian cats) were collected from cat breeders in Sweden, the population in which rdAc was first described (Narfström 1983; Narfström 1985b). Forty-three of the individuals had been clinically evaluated for rdAc status; pedigrees and ages were obtained for all cats. DNA was extracted from buccal swab samples using Qiagen kit DNeasy Tissue Kit (Valencia, CA). PCR was performed, using primers listed in Table A4, using touchdown PCR and DNA sequencing as described above. A population genetic survey of the *CEP290* mutation was conducted in 22 cat breeds, Abyssinian (from cat breeders in the United States) (16), American Shorthair (2), Birman (2), British Shorthair (2), Burmese (2), Chartreux (2), Colorpoint Shorthair (2), Devon Rex, Egyptian Mau (2), Havana (2), Bobtail (1), Maine Coon Cat (2), Manx (2), Norwegian Forest Cat (2), Ocicat (2), Persian (2), Ragdoll (2), Russian Blue (2), Siamese (2), Sphynx (2), Turkish Angora (2), and Turkish Van (2) from DNA stocks in the LGD collection (O'Brien et al. 2002).

Results

We initially sought to identify the gene responsible for rdAc using a candidate gene approach. Thirty-nine loci or genomic regions previously implicated in human RP or retinal degeneration (Table A1) were examined for linkage to disease phenotype with tightly linked STRs that were either previously mapped in the cat genome (Menotti-Raymond et al. 2003,a,b) or generated in silico from the domestic cat whole-genome sequence traces http://www.ncbi.nlm.nih.gov/BLAST/tracemb.shtml. Our failure to detect significant linkage of rdAc to any of the candidate genes/genomic regions stimulated a genome scan in the 122-member (79 genotyped) backcross pedigree. Significant linkage (LOD = 15.5, $\Theta = 0.00$) was detected between rdAc and STR marker FCA652 on chromosome B4 (Table 1) (Menotti-Raymond et al. 2003a). Additional gene-associated STRs were developed for fine mapping in silico from the domestic cat whole-genome sequence trace archive database to define the region of zero recombination with disease phenotype. The bounding markers for the linkage interval included STRs in the cat ortholog of the *PAMCI* gene (PAMCI, peak score +13.07, $\theta = 0.02$) and an STR with conserved synteny to human chromosome 12 sequence at approximately 91.3 Mb (Human12:91.34, peak score +4.78, $\theta = 0.04$) (Table 1), an interval of approximately 7.6 Mb. The region exhibited conserved synteny to human chromosome 12; the maximal linkage interval spanned a region from approximately 84.75 to 91.34 Mb in human genome build 36 (Table 1). The region of conserved synteny in human build 36 has 18 named genes (http://www.ncbi.nlm.nih.gov/mapview/). Of these, 10 exhibited retinal expression in previous studies of mammals other than cats (Schulz et al. 2004) and 4 other genes we established with feline retinal expression (Table 2).

The recent sequencing of the domestic cat genome at 2-fold genome equivalents (Pontius JU, Mullikin JC, Smith D, Agencourt Sequencing Team, Lindblad-Toh K, Gnerre S, Clamp M, Chang J, Stephens R, Neelam B, Volfovsky N, Schäffer AA, Agarwala R, Narfström K, Murphy WJ, Giger U, Roca AL, Antunes A, Menotti-Raymond M, Yuhki N, Pecon-Slattery J, Johnson WE, Bourque G, Tesler G, O'Brien SJ, unpublished data) was an important resource in the fine mapping and characterization of the causative mutation for rdAc. A female Abyssinian cat, *Cinnamon*, was selected for the genome project from over 1000 purebred cats, prescreened for low STR and SNP variability (He = 0.2), which is a critical attribute for accurate whole-genome sequence assembly. *Cinnamon* is also an affected rdAc individual.

Table 1. STR and SNP markers utilized in fine mapping of rdAc

Marker[a]	LOD	Theta	Cat chromosome B4 RH position (cR)	Dog (Chr:position, Mb)	Human May 2006 (Chr:position, Mb)
FCA864	2.03	0.24	873.9	10:9.62	12:62.17
FCA867	3.77	0.21	926.4	10:8.26	12:60.71
FCA051	1.66	0.08	943.4	Not found	Not found
FCA044	2.42	0.17	956.3	10:15.29	12:69.08
FCA868	3.02	0.15	981.5	10:12.46	12:65.68
PAMCI	13.07	0.02	—	15:30.31	12:84.75
Dog 15:30.47	4.83	0	—	15:30.47	12:84.90
Dog 15:30.54	14.89	0	—	15:30.54	12:85.00
Dog15:30.62	5.13	0	—	15:30.62	12:85.07
Dog15:30.97	15.53	0	—	15:30.97	12:85.49
Dog15:31.13	15.53	0	—	15:31.13	12:85.67
Dog15:31.34	15.53	0	—	15:31.34	12:85.92
FCA652	15.5	0	1025.6	15:31.77	12:86.48
KITLG	15.31	0	—	15:32.56	12:87.39
TUWD12	0.87	0	—	15:33.40	12:88.33
FCA210	7.70	0	1045.9	15:34.20	12:89.23
Human12:89.62	15.53	0	—	15:34.48	12:89.62
Dog 15:34.50	7.44	0	—	15:34.50	12:89.64
Dog 15:34.51	8.91	0	—	15:34.51	12:89.64
Dog 15:34.56	8.91	0	—	15:34.56	12:89.72
LUM	7.76	0	—	15:34.80	12:90.00
Human12:90.63	8.47	0	—	15:35.31	12:90.63
Human12:91.34	4.78	0.04	—	15:35.99	12:91.34
Human12:91.64	7.44	0.05	—	15:36.10	12:91.64
FCA735	1.87	0.23	1064.2	15:39.98	12:96.24
FCA871	0.53	0.31	1116.8	10:30.68	22:35.59
FCA520	3.20	0.12	1129.0	10:31.62	22:34.37

[a] Markers with FCA prefixes are previously published microsatellites. *KITLG*, *TUWD12*, and *LUM* are SNP'S discovered in the cat in this study and the remaining markers are new microsatellites identified from the cat 2X whole-genome sequence. Primer names correspond to position in human or dog genome. CR, centiray; RH, radiation hybrid.

Sequences of candidate loci in the region of zero recombination were queried from the cat whole-genome sequence traces in order to identify a causative mutation, design primers to complete partial sequences of candidate genes, and amplify and sequence homologous regions in unaffected individuals. Additionally, we began sequencing cDNAs for candidate genes, which were generated from RNA extracted from retinal neuronal and pigment epithelial tissues from both normal and affected rdAc cats. Full-length cDNA was generated for four genes (*DKFZp434N2030*, *DUSP6*, *ATP2B1*, *LUM*); partial cDNA was generated for two genes (*WDR51B*, *PAMCI*), and genomic DNA was generated for the entire coding region of the two genes, *NTS* and *MGAT4C* (Table 2). In all cases, we found no polymorphisms between affected and unaffected individuals, which segregated with rdAc. We ultimately focused on *CEP290* with report of its implication in retinal degeneration in humans (Sayer et al. 2006; Valente et al. 2006) and the *rd16* mouse (Chang et al. 2006).

Analyses of cDNA and genomic DNA demonstrated the presence of a mutation in intron 50 (IVS50 + 9T>G) (Figure 1) (den Dunnen and Antonarakis 2001) of *CEP290* that cosegregated with rdAc. The mutation creates a strong canonical splice donor site 4 bp downstream of the noncanonical splice donor site (Burset et al. 2000). The sequence of cDNA, generated from affected and unaffected individuals, demonstrated that the newly generated canonical splice site in affected individuals is utilized to the exclusion of the noncanonical splice site (at least, within the limits of our detection, Figure 1). This new pattern of splicing of intron 50 in affected individuals results in a 4-bp insertion and frameshift in the retinal neuronal *CEP290* transcript, with subsequent introduction of a stop codon immediately downstream (Burset et al. 2000) (Figure 1). The resulting transcript, if translated, would generate a peptide truncated by 159 amino acids (Figure A1), eliminating the most 3′ domains, KIDV and VI (Sayer et al. 2006; Valente et al. 2006). The mutation segregated in a homozygous state in all affected rdAc cats in the pedigree. Additionally, all phenotypically normal rdAc cats demonstrated the presence of at least one wild-type intron 50 splice donor. The wild-type *CEP290* retinal neuronal product was determined from cDNA analysis generated from two unrelated wild-type cats (GenBank accession no. EF028068).

The entire coding region of the *CEP290* cDNA was sequenced in one rdAc-affected and one rdAc-unaffected cat. We also partially sequenced the cDNA in a second unaffected cat. We observed five SNP's and the 4-bp insertion, which putatively is responsible for the rdAc mutation in affected individuals (Table 4). Four of the SNPs are synonymous in nature. One of the SNPs would result in a nonsynonymous substitution that would be conservative in nature. The affected cat was homozygous for the presence of the 4-bp

Table 2. Genes in region of zero recombination based on conserved synteny with human

	Candidate region (Position on Hsa 12) (Mb)	Function	Exons	Known retinal expression	Feline-tested retinal epression	rdAc sequenced	Assembled from cat WGS
PAMCI	84.73	Secretory trafficking	2	No	Yes	cDNA partial (15 of 435 aa sequence)	
NTS	84.77	Neuropeptide	4	Yes		Genomic DNA[a]	
MGAT4C	85.03	Mannosyl (alpha-1,3-)-glycoprotein beta-1,4-*N*-acetylglucosaminyltransferase, isozyme C	14	Yes			Entirely assembled
LOC400058	86.66	Mouse ring finger protein/human pseudogene	8	No			
FLJ35821	86.9	Hypothetical	13	No	Yes		
DKFZp434N2030	86.94	Hypothetical	7	No	Yes	cDNA entire	
CEP290	86.95	Centrosomal protein	54	Yes	Yes	cDNA entire	
TMTC3	87.05	Unknown	14	No	Yes		
KITLG	87.42	Hematopoetic growth factor	10	Yes		partially assembled	
DUSP6	88.25	Protein phosphatase	3	Yes	Yes	cDNA entire	
WDR51B	88.35	Messenger RNA processing	14	Yes	Yes	cDNA partial[b]	
CENPC2	88.42	Centromere protein C2	11	No			
GALNT4	88.43	Acetylgalactosaminyltransferase	1	Yes			
ATP2B1	88.50	Transmembrane Ca^{++} pump	21	Yes	Yes	cDNA entire	
LOC338758	88.65	Hypothetical	2	No			
LOC643153	89.83	Hypothetical	4	No			
C12ORF37	89.87	Hypothetical	5	No			
C12ORF12	89.90	Hypothetical	1	No			
EPYC	89.91	Epiphycan	7	No			
KERA	89.95	Keratan sulfate proteoglycan	3	No			
LUM	90.00	Leucine rich proteoglycan	3	Yes	Yes	cDNA entire	
DCN	90.09	Pericellular matrix proteoglycan	10	Yes			

Hsa, human; WGS, whole-genome sequence.

[a] Complete coding assembled from amplification of 4 exons.

[b] Missing nucleotides encoding first 122 aa of 477 aa protein.

insertion, whereas the unaffected cats were both homozygous for the absence of the insertion. In all cases, the affected cat was homozygous for the SNPs observed. If one of these other SNPs was causative of the rdAc mutation, we would predict there would likely be a fixed difference between the affected cat and the unaffected cat because the unaffected cat was a nonbreed cat with normal vision which is unlikely to be a carrier of rdAc. There was only one case where there was a fixed difference between the affected and unaffected cat and that was a synonymous substitution that was downstream of the putative mutation.

The prospect that the allele we define is not the causal SNP is an ongoing issue in disease gene identification by linkage and by association analysis and that a mapped locus will be tracking another causal SNP by linkage disequilibrium, particularly in an inbred cat. However, we believe that we have provided compelling reasons to demonstrate that *CEP290* (IVS50 + 9T>G) is the causal mutation. We cannot formally exclude that there is another noncoding SNP in LD with our candidate traveling in complete LD that may also contribute to the phenotype.

We have examined the frequency of the rdAc mutation (IVS50 + 9T>G) in a sample set of 79 Abyssinian and Somali (long-haired Abyssinian) cats provided by cat breeders in Sweden (Table 3), the population where rdAc was first described (Narfström 1985b). Twenty-one individuals were observed to be heterozygous for the rdAc allele and 58 individuals were homozygous for the wild-type allele. No rdAc homozygotes were observed in the sample set. The frequency of the rdAc allele is therefore 0.133 in our sample set of Swedish Abyssinian/Somali cats. Forty-four of these cats have been clinically evaluated for rdAc and none were determined to be positive for rdAc; 10 of the cats are greater than 3 years of age, the age at which rdAc can be established with confidence (Narfström 1985a).

We have additionally examined the frequency of the rdAc allele in a sample set of Abyssinian and Somali cats obtained from cat breeders in the United States, and a second sample set of 39 cats representing 20 different breeds, selected as representative of genetic diversity across cat breeds (Table 3) (Menotti-Raymond M, David VA, Pflueger SM, Lindblad-Toh K, O'Brien SJ, Johnson WE, unpublished data). In the Abyssinian and Somali samples (of US origin), the rdAc frequency was 0.056 and 0.012 in our population genetic survey of 20 cat breeds. One individual, an Ocicat, demonstrated the presence of a single rdAc allele. Ocicats are

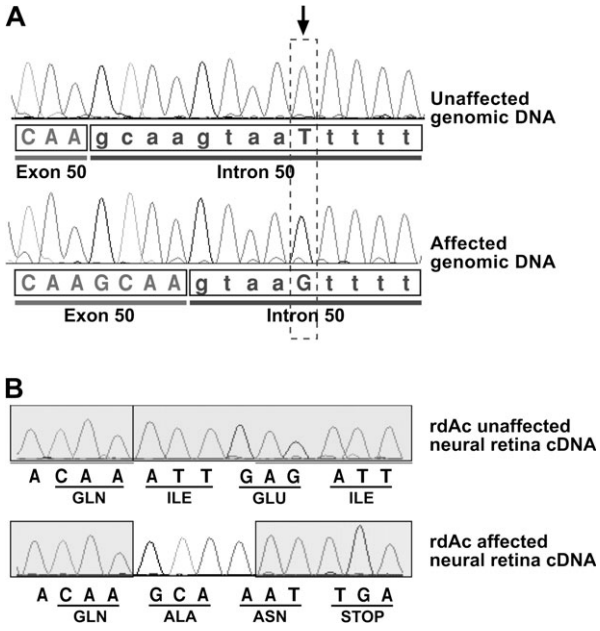

Figure 1. (**A**) Electropherograms of genomic DNA of *CEP290* sequenced in affected and unaffected individuals of exon 50/intron 50 junction. Arrow indicates position of SNP in intron 50, which uncovers a canonical GT splice donor site, resulting in alternative splicing in affected individuals. Exon 50 and Intron 50 (blue letters) nucleotides were identified by cDNA sequence analysis (see in color as Supplementary Figure 1). GenBank accession number for feline CEP290: EF028068. (**B**) Electropherograms of cDNA for CEP290 3′ region of exon 50 generated from neural retinal tissue in affected and unaffected individuals. Alternative splicing in affected individuals results in a frameshift and introduction of a premature STOP codon.

a recently developed hybrid breed, which originated from interbreeding of Abyssinian, Siamese, and American Shorthair cats (http://www.cfainc.org/breeds/profiles/ocicat.html).

The CEP290 protein is highly conserved in mammals, as reported previously (Chang et al. 2006; Sayer et al. 2006). The cat displays 92.1% and 95.6% amino acid sequence homology to CEP290 of human and dog, respectively (Figure A1). Fifty nucleotide differences were observed between the cat and dog CEP290 GenBank sequences. Fifty-six percent of the CEP290 coding sequence was recovered from the domestic cat 2X whole-genome sequence traces; however, the rdAc defining mutation was not included in this data. No SNPs were observed in a comparison of the 2X whole-genome sequence traces available for CEP290 and the affected individual we sequenced, as would be expected in an affected individual in the genomic region of a recent mutation that demonstrates an AR mode of inheritance.

Discussion

The identification of the feline *CEP290* mutation attests to previous reports of the importance of this gene in retinal function: 2 human diseases, Joubert syndrome, a syndromic retinopathy with variable neurological and renal involvement (Sayer et al. 2006; Valente et al. 2006), and Leber's congenital amaurosis (LCA), an AR, nonsyndromic early-onset retinopathy (den Hollander et al. 2006), as well as an early-onset retinal dystrophy in the *rd16* mouse (Chang et al. 2006), are attributed to mutations in the *CEP290* gene.

Rod photoreceptors arise from a class of organelles known as primary cilia, which have gained attention in the last few years due to their implication in several human hereditary diseases, including polycystic kidney disease and RP (reviewed in Eley et al. 2005; Badano et al. 2006; Satir and Christensen 2007). It is now well illustrated that primary cilia act as sensory organelles projecting from many eukaryotic cells (http://www.primary-cilium.co.uk/), which respond to a spectrum of sensory stimuli and initiate signaling pathways, enabling the cell to respond to changes in the external environment (Singla and Reiter 2006; Satir and Christensen 2007). The outer segments of the rod photoreceptor matures from a primary cilia by synthesis and transport of specialized phototransduction proteins and lipids into the distal portion of the cell by a specialized ciliary transport process called intraflagellar transport (IFT), a kinesin-II based movement of multisubunit protein particles along flagellar microtubules (Pazour and Rosenbaum 2002). In the mature rod, the remaining physically distinguishable part of the primary cilium is the connecting cilium, which acts as the conduit for the trafficking of proteins and lipids synthesized in the inner segment into the outer segments, where the phototransduction process occurs (Pazour and Rosenbaum 2002).

In the *rd16* mouse model, CEP290 localizes to the connecting cilium and colocalizes with other proteins critical in IFT, including the RP GTPase regulator (RPGR), a gene that causes about 2/3 of X-linked RP and also an undetermined percentage of X-linked cone-rod dystrophies (Hamel 2007). As the rod photoreceptor discs are in a constant state of regeneration, and thus undergo an extremely high rate of protein turnover, a fully functional IFT system is critical for the maintenance of the rod photoreceptors (Pazour and Rosenbaum 2002; Badano et al. 2006). Mutations in other IFT proteins have previously been demonstrated as causative of retinal degeneration (Marszalek et al. 2000; Pazour et al. 2002; Mykytyn et al. 2004; Nishimura et al. 2004). The *rd16* mice demonstrate a redistribution of specialized phototransduction proteins such as opsin and rhodopsin in the inner segment, leading Chang et al. to propose a ciliary trafficking role for the wild-type CEP290 product (Chang et al. 2006).

The rdAc cat offers additional support to these findings. In early stages of disease, affected individuals demonstrate a marked vacuolization in the basal part of the outer segment of the rod photoreceptor, near the connecting cilium, which is in the region of new photoreceptor disc formation (Narfström 1985b) (Figure 2). This finding suggests early abnormalities in the transport and distribution of phototransduction and/or structural proteins through the connecting cilia in the rdAc model, resulting in photoreceptor degeneration.

Several mutations are reported in *CEP290* that cause Joubert syndrome (Sayer et al. 2006; Valente et al. 2006); they are largely nonsense or frameshift mutations, which likely result in loss of function of CEP290. Extended pathology

Table 3. Population genetic survey of cat breeds for *CEP290* (IVS50 + 9T>G) genotype

	Sample size	rdAc status	Wild type	Carrier	rdAc IVS50 + 9T>G frequency
Swedish Abyssinian	56	Unaffected[a]	39	17	0.133[b]
Swedish Somali[b]	23	Unaffected[a]	19	4	0.133[b]
USA Abyssinian	16	ND	14	2	0.07
American Shorthair	2	ND	2		
Birman	2	ND	2		
British Shorthair	2	ND	2		
Burmese	2	ND	2		
Chartreux	2	ND	2		
Colorpoint Shorthair	2	ND	2		
Devon Rex	2	ND	2		
Egyptian Mau	2	ND	2		
Havana	2	ND	2		
Japanese Bobtail	1	ND	1		
Maine Coon Cat	2	ND	2		
Manx	2	ND	2		
Norwegian Forest Cat	2	ND	2		
Ocicat	2	ND	1	1	
Persian	2	ND	2		
Ragdoll	2	ND	2		
Russian Blue	2	ND	2		
Siamese	2	ND	2		
Sphynx	2	ND	2		
Turkish Angora	2	ND	2		
Turkish Van	2	ND	2		
Total	136				

ND, not determined.

[a] Affection status based on clinical exams.

[b] Frequency reported as incidence observed in Abyssinian and Somali (long-haired Abyssinian) as the breeds are interbred.

beyond retinal involvement is observed in Joubert-affected individuals in the cerebellum and kidneys (Sayer et al. 2006; Valente et al. 2006). In contrast, the rd16 mouse exhibits only retinal pathology, suggestive that the loss of the myosin-tail homology domain of CEP290 performs a retina-specific function (Chang et al. 2006). No pathology is observed in the rdAc cat beyond retinal involvement. The rdAc cat mutant CEP290 retains the myosin-tail domain of CEP290 (Figure A1), but the premature stop codon truncates the putative peptide, which would delete the more 3' KIDV and VI domains (Chang et al. 2006) (Figure A1), suggesting that these most terminal domains may also perform a retinal specific function. This suggests the hypothesis that mild mutations on the 3' end of *CEP290* may occur in human ARRP, for which the majority of patients (60%) (Wang et al. 2005), have no molecular diagnosis.

We observed a frequency of 0.14 for the rdAc allele in a sampling of Swedish Abyssinians and Somalis. When rdAc was first described in the Swedish population approximately 25 years ago (Narfström 1983), affected cats were relatively common. Since then, the breeders of Abyssinian and Somalis have made a concerted effort to have cats examined on an annual basis to identify affected individuals and thereby carriers in order to reduce the incidence of rdAc in the population. A lower incidence (0.07) of the rdAc allele was observed in a sample set of Abyssinian cats collected from breeders in the United States, which was not unexpected given that they are different breeding populations. In our survey of 20 additional cat breeds, selected to sample the range of genetic diversity in cat breeds (Menotti-Raymond M, David VA, Pflueger SM, Lindblad-Toh K, O'Brien SJ, Johnson WE, unpublished data), the rdAc allele was extremely rare (0.012) and observed only once in an Ocicat breed cat. The Ocicat is a recently developed hybrid breed, which was generated from interbreeding of Abyssinian,

Table 4. Polymorphism detection in the cDNA of *CEP290* in 2 rdAc-unaffected cats and 1 rdAc-affected cat

Polymorphism[a]	Sequence in 2 unaffected cats[b]	Sequence in rdAc-affected cat	Polymorphism effect
399Y[c]	T, TC	T	Synonymous
1080R	GA, A	G	Synonymous
3405R	GA, ND	G	Synonymous
3743Y	CT, ND	C	T = val/ C = ala
INS6957CAAG	No insertion, ND	CAAG inserted	Results in truncation
7005R	G, ND	A	Synonymous

ND, not done; only one unaffected cat was sequenced in this region of the cDNA.

[a] The number indicates the nucleotide position from the ATG start position in the *CEP290* cDNA.

[b] The unaffected cats were wild-type non-Abyssinian cats.

[c] The letter indicates the nucleotide polymorphism code.

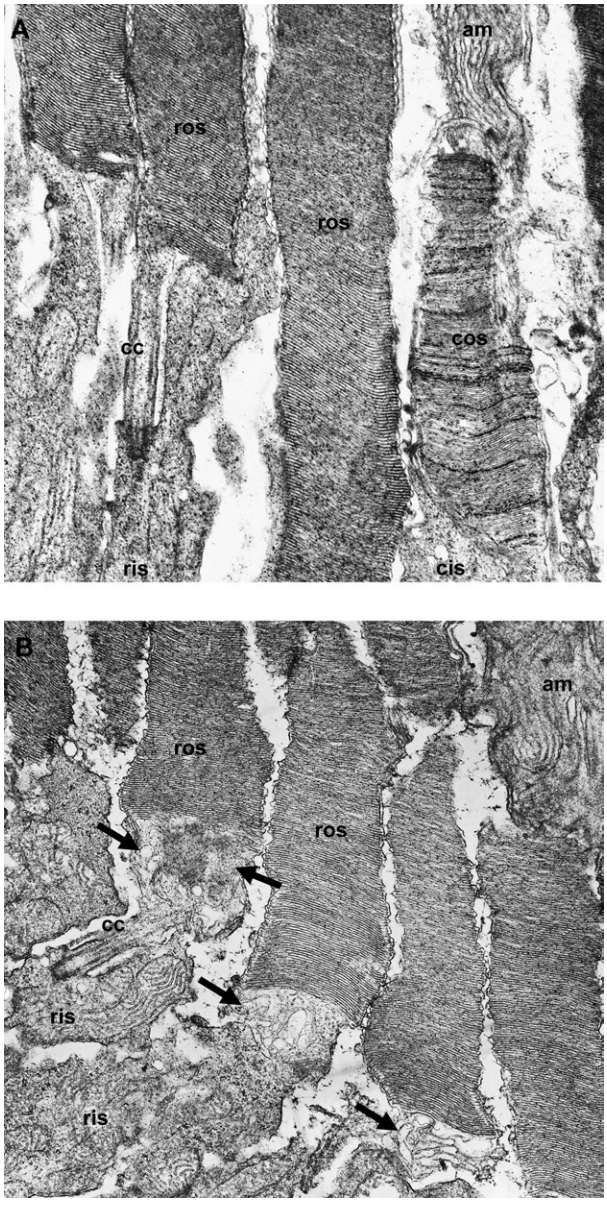

Figure 2. Electron micrographs of outer retina showing photoreceptor outer and inner segments of normal (**A**) Abyssinian cat and young affected (**B**) rdAc cat. Note abnormalities at the base of the rod outer segments near the connecting cilium in **B**; membranes are not formed as in the normal (**A**), instead there is vacuolization and degeneration (arrows) of membranes in the affected retina. Am, apical microvilli of the retinal pigment epithelium; ros, rod outer segments; ris, rod inner segments; cos, cone outer segments; cis, cone inner segments; cc, connecting cilium of the photoreceptor. Original magnification: 19152×.

American Shorthair, and Siamese individuals (http://www.cfainc.org/breeds/profiles/ocicat.html), thus allowing for the introduction of the rdAc allele into the Ocicat breed group.

Cats have historically been important models in neurophysiology and neuroanatomy, particularly with respect to visual function. The cat has a relatively large eye and retinal structures, facilitating physiological studies and surgical interventions. Pazour et al. report that the size of the mouse retina posed physical limitations in earlier studies that examined the trafficking of ciliary proteins in photoreceptor cells, leading to their use of a normal bovid eye (Pazour et al. 2002). The characterization of the gene defect in the rdAc model should facilitate physiological studies of the role of CEP290 in photoreceptor cells.

The eye is an ideal organ with which to examine the potential of gene replacement intervention; it offers a confined environment, which minimizes the potential of systemic effects, while maximizing the facility to target a gene vector and visualize its ability to reverse pathology (Bainbridge et al. 2006). Gene therapeutic interventions for retinal degenerative conditions have shown considerable progress in the past few years (Smith et al. 2003; Schlichtenbrede et al. 2004; Tschernutter et al. 2005; Bainbridge et al. 2006); the LCA dog model demonstrates a particularly promising intervention (Acland et al. 2001; Narfström et al. 2003). Clinical trials of rAAV-mediated gene therapy for patients with LCA have recently been approved after the demonstration of long-term functional improvement in the LCA dog model following gene replacement of RPE65 (Narfström et al. 2005; Bainbridge et al. 2006). Large animal models have the additional potential to evaluate the efficacy of gene therapeutic interventions given aspects of safety and background genetic heterogeneity similar to humans and, additionally, enable critical long-term studies (Casal and Haskins 2006).

A mutation in *CEP290* is also implicated in human LCA and may be responsible for up to 21% of cases (den Hollander et al. 2006). The identification of rdAc as a model of human photoreceptor degeneration offers considerable promise in developing gene-based therapies for humans affected with severe retinal blinding disease.

The mapping and characterization of the causative mutation for rdAc is additionally important validation of the cat 2X whole-genome sequencing effort (Pontius JU, Mullikin JC, Smith D, Agencourt Sequencing Team, Lindblad-Toh K, Gnerre S, Clamp M, Chang J, Stephens R, Neelam B, Volfovsky N, Schäffer AA, Agarwala R, Narfström K, Murphy WJ, Giger U, Roca AL, Antunes A, Menotti-Raymond M, Yuhki N, Pecon-Slattery J, Johnson WE, Bourque G, Tesler G, O'Brien SJ, unpublished data). The availability of sequence traces allowed us to develop additional STRs for fine mapping in the implicated genomic region and to develop primers from cat sequence for amplification of cDNA in candidate genes. Given the availability of a large number of naturally occurring cat models for a variety of human genetic disorders, it is likely that the newly available 2X genome sequence (Pontius JU, Mullikin JC, Smith D, Agencourt Sequencing Team, Lindblad-Toh K, Gnerre S, Clamp M, Chang J, Stephens R, Neelam B, Volfovsky N, Schäffer AA, Agarwala R, Narfström K, Murphy WJ, Giger U, Roca AL, Antunes A, Menotti-Raymond M, Yuhki N, Pecon-Slattery J, Johnson WE, Bourque G, Tesler G, O'Brien SJ, unpublished data), and upcoming sequencing of the cat genome at 7X equivalents, will play a substantial role in our identification of the genes underlying these disorders and contribute to our understanding of human genetics and disease.

Supplementary Material

Supplementary Figure 1 and Appendix can be found at http://www.jhered.oxfordjournals.org/.

Acknowledgments

We thank Dr Guo Kui Pei and Lisa Maslan who generated STR genotype data and sequence data on the ABI3100 and 3700, respectively, and Matthew Barr and Kristina Bajema who provided technical assistance. We would also like to thank Gunilla Naesenius for coordinating the collection of Abyssinian/Somali samples from cat breeders in Sweden. The Foundation Fighting Blindness (K.N.) and the National Cancer Institute have funded this project. This research was supported in part by the Intramural Research Program of the National Institutes of Health and the National Library of Medicine.

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Received March 3, 2007
Accepted March 19, 2007

Corresponding Editor: Roger Reeves